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## Document heading

Effects of ethanol extract of leaves of *Helianthus annus* on the fecundity of Wistar ratsEjebe Daniel Emamuzo<sup>1\*</sup>, Siminialayi Iyeopu Miniakiri<sup>2</sup>, Emudainohwo Joseph Oghenebrorie Tedwin<sup>1</sup>, Kagbo Hope Delesi<sup>2</sup>, Amadi Precious<sup>3</sup><sup>1</sup>Department of Pharmacology, Delta State University Abraka, Nigeria<sup>2</sup>Department of Pharmacology, University of Port Harcourt, Nigeria<sup>3</sup>Institute of Science Laboratory and Technology, Delta State University, Abraka Nigeria

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## ABSTRACT

**Objective:** To evaluate the effects of ethanol extract of leaves of *Helianthus annus* (*H. annus*) on the fecundity of Wistar rats. **Methods:** Forty (20 male and 20 female) Wistar rats, grouped into control, ethanol extract treated rats and untreated, were used for the study. Treated rats had 0.5 g/kg of ethanol extract orally for 2 weeks; control rats had 5% alcoholic water (solvent); and untreated rats had their normal feed and clean drinking water. Following treatment, the animals were sub-grouped into 5 mating groups to observe the coital frequency, pregnancy rate and average number of pups per group in pairs. **Results:** The results showed that coital frequency was unaffected by the extract treatment but pregnancy rate and number of pups per rat and per group were reduced significantly in groups II, III and IV compared to those of group I and V rats which were not treated with the ethanol extract of leaves of *H. annus*. **Conclusions:** The histodegenerative in the gonads reportedly induced by this ethanol extract in previous studies may be responsible for the reduced fecundity observed in treated adult rats.

## 1. Introduction

Long before the advent of orthodox medicines, different human populations had their various traditional medical systems which also had their unique means of preventing conception when it was not wanted by couples. The tools reportedly used in these traditional medical systems are known to include herbs, animal products, minerals and rituals, of which herbs are the most commonly used[1,2].

Several herbal medicines obtained from different parts of plants have been reported to have been used for contraception but only few have anti fertility effects confirmed by experiments carried out on both humans and laboratory animals. Some of the validated anti-fertility plants with potential for contraception include: neem oil[3,4], *Montenoea tormentosa*[5,6], *Montenoea frutescens*[7], *Malvaviscus conzatti*[8], and racemic gossypol obtained from cotton seed[9,10].

*Helianthus annus* (*H. annus*), commonly called sunflower, is an annual perennial plant belonging to the family of

Asteraceae which is distributed worldwide[11]. Its folklore medicinal uses in many ways have been documented[12]. In our previous studies on the ethanol extract of the leaves of *H. annus* we reported its oral LD<sub>50</sub> in rats as 14 g/kg of animal weight as well as the presence of steroids, tannins, glycosides, saponins, reducing sugars, and carbohydrates on initial phytochemical screening[13]. Our previous investigation on the effects of the ethanol extract of the leaves on the histology of the testes, blood level of some reproductive hormones and epididymal sperm properties in Wistar rats suggested the existence of some anti-fertility effects[14,15].

This study is designed to ascertain if the observed anti-spermatogenic effects of the ethanol extract of the leaves of *H. annus* in our previous study would translate into actual contraceptive outcome and to find out if any such anti-fertility effects would be present in female rats. It determined the effects of the ethanol extract of *H. annus* on the fecundity of Wistar rats.

## 2. Materials and methods

## 2.1. Plant collection and preparation of the extract

The fresh leaves (2 kg) of the wild variety of the plant were harvested in Abraka, South of Nigeria in April 2009

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and authenticated by Mr. Ebigwa E., a taxonomist of Botany Department of the Delta State University, Abraka.

The leaves were washed, air dried and then ground to powder. 100 g of the powder was measured and soaked in 500 mL of absolute ethanol (BDL, England) for 24 hours. The mixture was then filtered using Watman 2.0 filter paper. The obtained filtrate was evaporated to dryness in a rotary evaporator at 40 °C.

## 2.2. Preparation of stock solution

The stock solution was prepared by dissolving 10 g of the extract in 100 mL of 5% alcoholic water to give a stock concentration of 0.10 g/mL.

## 2.3. Animal husbandry

Twenty adult male and twenty adult female rats weighing between 100–150 g were procured from the breeding colony of the College of Health Sciences, Delta State University, Abraka and were housed in plastic cages within the animal facility of the Faculty of Basic Medical Sciences of the University. The animals were allowed to acclimatize for 2 weeks before commencement of the experiment and throughout they were allowed free access to clean drinking water and standard rat chow.

## 2.4. Animal experiment

The animals were weighed and divided into control groups of eight male and eight female rats which received 5% alcoholic water treatment; extract treated group made up of eight female and eight male rats which received 0.5 g/kg of the ethanol extract of *H. annuus* leaves dissolved in 5% alcoholic water and a set of four female and four male rats which were administered neither 5% alcoholic water nor the ethanol extract. The male rats were kept in separate cages from their female counterparts in each group during the treatment phase. The animals were administered with corresponding test substance orally, using medicut intravenous cannula as an improvised oral cannula daily for 14 days<sup>[16]</sup>. Following completion of treatment, the rats were weighed again before a male and a female rat were randomly selected from the different treatment groups into a separate cage where they were allowed to mate continuously for another 14 days<sup>[17]</sup>. The rat couples were further sub-grouped into: group I: control females (4) that were individually housed with a control male (4); group II: control females (4) that were individually housed with an ethanol extract treated male (4); group III: ethanol extract treated females (4) that were individually housed with a control male (4); group IV: ethanol extract treated females (4) that were individually housed with an ethanol extract treated male (4); Group V: non treated females (4) that were individually housed with a non treated male (4).

During the 14 days of continuous mating, each female rat was examined at the same time every day for the presence of vaginal sperm plug as an indirect evidence of mating in the previous 24 hours. The frequency of this sign was recorded as the coital frequency of the different rat couples<sup>[18]</sup>.

At the end of the 14th day of continuous mating, the female rats were isolated from their male partners and observed for pregnancy for another 23 days on the assumption that if fertilization had occurred on day 14 the required gestational

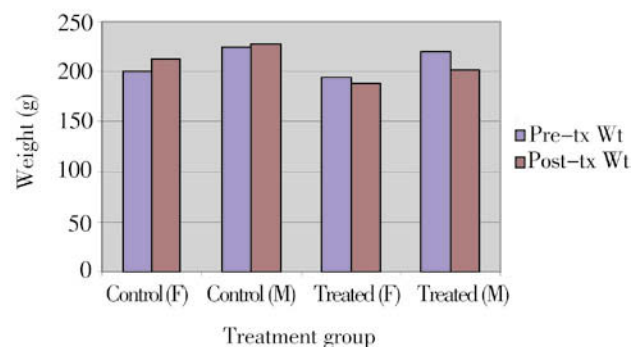
period is another 23 days<sup>[19]</sup>. The number of pregnancies, the number of pups delivered by each female rat in different groups were recorded and used to compute the average number of pups and percentage of pregnancy rate per group.

## 2.5. Statistical analysis

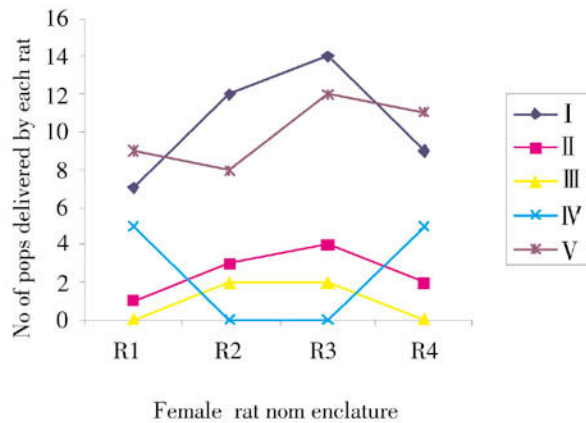
The results were fed into the spread sheets of Microsoft Excel 2003 computerized software and analyzed for statistical significance using the Student's *t*-test of two different means assuming unequal variances. *P*-values less than 0.05 were considered to be statistically significant.

## 3. Results

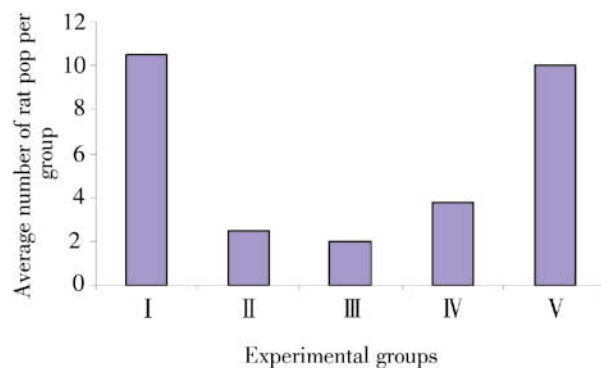
The results showed that post-treatment mean bodyweights were lower than the pretreatment mean bodyweights in the extract treated rats of both sexes, while the control rats gained weight (Figure 1). But changes in weights were however statistically insignificant ( $P>0.05$ ). The number of pups delivered by females rats of group II, III and IV in which either one or both member(s) were treated with the ethanol extract of *H. annuus* were fewer than those of groups I and V in which non of the coupled rats received any extract (Figure 2). In fact two rats (R1 and R4) in group III and two in group IV (R2 and R3) had no pups despite having continuously mated like the other females. Figure 3 further showed that the average number of rat pups delivered in each group were lower in groups II, III and IV compared to those of groups I and V. Figure 4 showed that while all the female rats in groups I and V got pregnant (100% pregnancy rates), only 50% of the female rats in groups II, III and IV, where either one or both members of the coupled rats had received the extract treatment, got pregnant. Effects of ethanol extract of *H. annuus* leaves on the coital frequency of male and female Wistar rats cohabited in pairs for an observational period of 2 weeks were listed as following (Mean $\pm$ SEM): Group I, 4.25 $\pm$ 0.48 (4); group II, 3.75  $\pm$ 0.75 (4); group III 3.75 $\pm$ 0.48 (4); group IV, 4.25 $\pm$ 0.48 (4); group V, 4.5  $\pm$ 0.29 (5). Comparison of mean coital frequencies between group I and other groups were not significant different ( $P>0.05$ ). The coital frequencies for each couple of male and female rats, estimated indirectly by confirmation of the presence of vaginal sperm plug in the female rats, were shown in Figure 5.



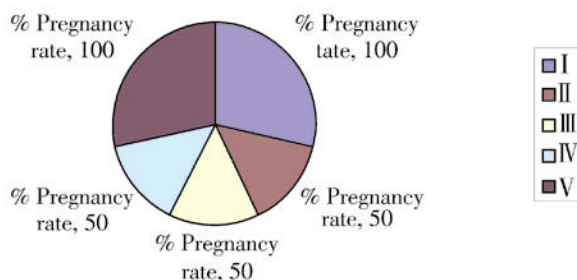
**Figure 1.** Effect of *H. annuus* leaf extract on bodyweight of Wistar rats. Mean  $\pm$  SEM, *n*= 4, (F) Female, (M)–Male, Pre-tx Wt(pretreatment weight), Post-tx Wt (Post treatment weight).



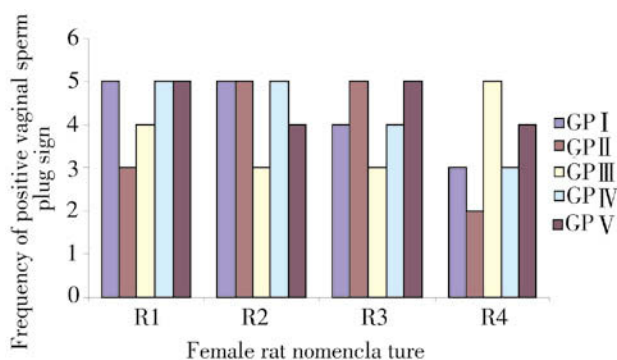
**Figure 2.** Effects of ethanol extract of *H. annus* on the number of pups delivered per index pregnancy by Wistar rats. R1,2,3,4 (Rat 1,2,3,4).



**Figure 3.** Effect of ethanol extract of *H. annus* leaves on the average number of pups per group of Wistar rats. Mean,  $n = 4$  female rats.



**Figure 4.** Effect of ethanol extract of *H. annus* leaves on the frequency percentage of viable pregnancy outcome in Wistar rats.  $n = 4$  female rats.



**Figure 5.** Effects of ethanol extract of *H. annus* leaves on libido of Wistar rats. R1,2,3,4 (Rat 1,2,3,4).

## 4. Discussion

The results showed that post-treatment mean bodyweights were lower than the pretreatment mean bodyweights in the extract treated rats of both sexes, while the control rats gained weight. The changes in weights were however statistically insignificant ( $P > 0.05$ ) and this observation was in line with our previous finding<sup>[15]</sup>. The number of pups delivered by female rats of group II, III and IV in which either one or both member(s) were treated with the ethanol extract were fewer than those of groups I and V in which non of the coupled rats received any extract. In fact two rats (R1 and R4) in group III and two in group IV (R2 and R3) had no pups despite having continuously mated like the other females. This observation points to some reduction in the fertility of these rats. The average number of rat pups delivered in each group were lower in groups II, III and IV compared to those of groups I and V. Besides, all the female rats in groups I and V got pregnant (100% pregnancy rates), only 50% of the female rats in groups II, III and IV, where either one or both members of the coupled rats had received the extract treatment, got pregnant. This observation further gives some credence to the existence of contraceptive effects in this extract. The observation that the coital frequencies for each couple of male and female rats, estimated indirectly by confirmation of the presence of vaginal sperm plug in the female rats during the 14-day period of continuous mating, ranged from 2 to 5 for groups I to V with some of the highest coital frequencies observed in rats in the extract treated groups II and III, suggests that the extract could not have reduced the fertility and fecundity of the rats by inhibition of libido. The results also showed that the average coital frequency for each group rounded to the nearest whole number ranged from 4 to 5 days during the two weeks of continuous cohabitation of the female and male rats and these rates were not significantly different ( $P > 0.05$ ) between the groups of coupled rats, further suggesting that a depreciation of the libido in rats was unlikely to have contributed to any reduced fertility and fecundity in rat couples exposed to the ethanol extract. The results also suggest that the extract has anti-fertility effects on ethanol extract treated female rats that were mated with control male rats. However a combined anti-fertility action was not observed as there were no differences in the number of pups per rat, average number of pups per group, number of pregnancy per group (% pregnancy rates) and coital frequencies, when group IV was compared with groups II and III.

The above observations further corroborate the conclusion from previous studies that suggested the possible existence of contraceptive potentials in the ethanol extract of the leaves of *H. annus*<sup>[13]</sup>. The underlying mechanism of this action as suggested in earlier studies could be direct toxicity on the testes or the hypothalamo-pituitary-gonadal axis<sup>[13]</sup>. However epididymal and sertoli cell toxicity could also translate into anti-spermatogenic effects<sup>[19,20]</sup>. In mice, neonatal exposure to dietary phytoestrogens has been reported to lower sperm concentration and plasma testosterone<sup>[21,22]</sup>; sertoli cell number<sup>[21]</sup>, gene expression<sup>[23]</sup> and subsequent infertility<sup>[24]</sup>. Similar structural and functional changes have been reported in adult rats<sup>[25]</sup>. If the steroids in the ethanol extract can be shown to be

phytosteroids, it could provide another explanation of the mechanism of its anti-fertility action. It appears that ethanol extract produces its anti-fertility effects on males and females alike.

This study concludes that extracts of the leaves of *H. annuus* reduced the fertility and fecundity of rats, it also reduced the fertility in treated female Wistar rats that were mated with untreated control male rats.

Further investigations to determine the mechanism(s) of action of the extract and the nature of the active principal(s) responsible for its noted effects need to be conducted.

## Conflict of interest statement

We declare that we have no conflict of interest.

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